IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 10 Ser. I (October 2022), PP 08-14
www.iosrjournals.org

Phytochemical study, determination of the antioxidant and antimicrobial activities of the bark and root extracts of *Bridelia micrantha*, a plant widely used in traditional African medicine

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Abstract: *Bridelia micrantha is a plant traditionally used in tropical Africa to treat a wide range of human and animal diseases. The present study aims to highlight the presence or not of some families of chemical compounds through phytochemical screening, to evaluate the antioxidant and antimicrobial activities of the different extracts from the barks and roots of Bridelia micrantha. Sequential macerations with solvents of increasing polarity (hexane, ethyl acetate, ethanol, and water) were carried out. The highest extraction rate of 50 % is obtained with crude ethanol. The antioxidant activity of the fractions and of the crude ethanolic extract were evaluated by the DPPH˙ method. The 50% inhibitory concentrations (IC50) vary from 1.1 ± 0.0 mg/mL to 31 ± 1.7 mg/mL (respectively for the ethanolic and ethyl acetate fractions) for barks and 0.17 ± 0.00 mg/mL to 19.40 ± 0.14 mg/mL for roots. The ethanol fraction of the roots has the highest antioxidant capacity with an IC50 of 0.17 ± 00 mg/mL. The disc diffusion method was used to study the sensitivity of the extracts on bacterial strains (Escherichia Coli ATCC25922, Enterococcus faecalis ATCC 29213, Staphylococcus aureus ATCC 29212, Pseudomonas and Candida Albican ATCC24433) and to evaluate their antimicrobial activity on these same strains. The results showed that the ethanol fraction of the roots exhibits the best antimicrobial capacity with an MIC of 1.875 mg/mL. The conclusions of this study provide scientific evidence that contributes to the enhancement of Senegalese flora.*

Keywords*: Bridelia micrantha, antioxidant activity, antimicrobial activity*

Date of Submission: 14-10-2022 Date of Acceptance: 30-10-2022

I. Introduction

Humans have always carried a greater number of indigenous bacteria than those contained in their own cells. The skin, the respiratory and gastrointestinal tracts are the entry routes for the introduction of exogenous bodies into the body. It is established that pathogenic bacteria can survive in air, soil and water for long periods of time and their persistence in the environment leads to an increased risk of infection in human and animal hosts [1]. Although the vast majority of bacteria are harmless or beneficial, a good number of them are pathogenic, thus causing disease on a global scale. This is the example of *Staphylococcus aureus*, *Shigella sonnei*, *Salmonella enterica serovaTyhimurium*, and *Helicobacter pylori* which are medically important pathogens causing potentially fatal infections such as pneumonia, meningitis and nosocomial infections [2].

The public health problem is one of the major concerns of poor populations and access to modern medicines is practically impossible. Consequently, man uses nature to heal himself, to feed himself, and to build houses. Thus, more than 80% of the world's population uses so-called traditional medicine to deal with health problems [3]. However, it should be noted that the population generally uses plants for therapeutic purposes without taking into account the dose, which can constitute a danger. Our study goes in the direction of establishing a bridge between traditional and modern medicine by evaluating the biological activity and the right dose necessary to minimize the risks.

The richness of the flora of Africa constitutes a major asset for the populations. Owing to the multitude of bioactive molecules in vegetable, plants are considered as a reservoir of medicine. Most of bioactive molecules are produced by plants as chemical defenses against predation and infection.Among these secondary

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metabolites, polyphenolic compounds are highly valued molecules in research centers because of their remarkable physiological and pharmacological activities [4]. *Bridelia micrantha* is one of the species found in most African countries. It is a plant traditionally used in tropical Africa and all over the world as a vermifuge, antiamoebic, antianaemic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal [5], antiinflammatory, antimalarial, antinociceptive, antiviral, hypoglycemic and against abdominal pain, diseases **cardiovascular**, gynecological and sexually transmitted [6]. Due to its popularity as a medicinal plant, *Bridelia micrantha* is sold as such in herbal medicine markets in Africa[7].

II. Material and methods

II.1. Plant material

The choice made on *Bridelia micrantha* is justified by an ethnobotanical survey coupled with a bibliographic study of the plants listed among certain traditional healers in Senegal. The plant material used consists of the bark and roots of *Bridelia micrantha* (Figure 1). The harvest of the different organs of this plant was carried out in October 2021 in the commune of Dioula Colon, located in south Senegal, with geographical coordinates 13°05' North and 14°49' West.The bark and roots of the plant were cut into small pieces and then dried in the shadowat 27°C.

Stem barkof*Bridelia micrantha*

Roots of *Bridelia micrantha*

Figure 1. Photograph of the different organs (Bark and Roots) of *Bridelia micrantha*.

II.2. Extraction procedures

The extraction of the secondary metabolites was carried out by cold maceration to avoid a possible degradation of the thermosensitive molecules present in the plant.

Plant material was extracted in solvents of increasing polarity. Indeed, a few grams of plant material are soaked in a few milliliters of hexane with a mass/volume ratio of 1/7, then stirred for 48 hours. After filtration, the marc is recovered, and the process is repeated successively with ethyl acetate, ethanol, and water. The filtrates obtained are concentrated using a rotary evaporator until the solvents are completely exhausted.

The crude ethanolic extracts were obtained by directly macerating a few grams of plant material in ethanol with a mass/volume ratio of 1/4 under magnetic stirring for 48 hours.

II.3. Phytochemical study

Phytochemical screening is a qualitative analysis based on precipitation or coloring reactions. The latter make it possible to define the presence or absence of secondary metabolites which may be found in a plant sample. In this work, the screening concerns the search for alkaloids, polyphenols, tannins, flavonoids, sterols, polyterpenes, coumarins and catechols. We tested the presence of these different chemical groups by referring to the techniques described in the work of Ronchetti and Russo [8,9]. Polyphenols and tannins were identified by the $FeCl_3$ test and Stiasny's reagent; flavonoids and catechols by reaction with cyanidin; sterols and polyterpenes by the Liebermann-Burchard test; coumarins by the ammonium hydroxide test and alkaloids by the Mayer test [10,11].

II.4. Antioxidant activity

The antioxidant test was performed using the $DPPH^{\cdot}$ method [12]. A stock solution of 40 mg/mL is prepared by dissolving 80 mg of dry extract in 2 mL of methanol. Then a concentration range was prepared with a 1/2 dilution factor. For each of these different concentrations, 0.2 mL is taken and introduced into test tubes and mixed with 7.8 mL of the purple methanolic solution of $DPPH^{\cdot}$, previously prepared. The tubes are stirred manually for a few seconds then incubated for 30 min away from light, the reading is carried out by a spectrophotometer at a wavelength of 517 nm, using methanol as a blank. Ascorbic acid is used as reference antioxidant.

The $DPPH^{\cdot}$ solution is obtained by dissolving 10 mg of $DPPH^{\cdot}$ in 250 mL of methanol in the dark after stirring for 30 minutes.

$$Scavengingactivity = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}$$

II.5. Antimicrobial activity

II.5.1. Bacteria sensitivity test

The disc diffusion method was used to test the sensitivity of bacterial strains to different extracts. The bacteria are inoculated on Muller-Hinton (MH) agar and on Slabouraud agar for *candida albican*[13]. From a bacterial culture of 18h to 24h, we prepare inocula equivalent to the Mac-Ferland standard 0.5 (106 bacteria per mL). On each Petri dish, a drop of the culture dilution (inoculum) prepared with a few micrograms of bacteria in a biological liquid (sodium chloride) was deposited and spread in tight streaks with a sterile swab, over the entire M-H agar as well as than on Sabouraud agar for *candida albican*.

Wells (Sabouraud) 0.7 cm in diameter were then placed on the upper layer of the agar medium, seeded with bacteria. The wells are impregnated with 100 µL of solution at a concentration of 30 mg/mL and the dishes are incubated in the oven at 37°C for 24 hours. Throughout the incubation period (24 hours at 37°C), the strain to be studied competes with the inhibitory effect of the plant extract. A circular zone of inhibition forms around the cupule when the strain is sensitive to the extracts and an absence of zones of inhibition if the strain is resistant. The extract is not effective if the diameter is less than 4.8 mm; effective if the diameter is between 4.8 and 9.6 mm and very effective if the diameter is greater than 9.6 mm.

II.5.2. Determination of minimum inhibitory concentrations (MIC)

The MIC is the lowest concentration of antibiotic capable of causing complete inhibition of the growth of a given bacterium, appreciable to the naked eye, after a given incubation period. The determination of the MIC was carried out for the extracts which showed zones of inhibition against the microorganism by the disk diffusion method [14]. Rectangular plates containing 96 wells were used, ranges of concentration were prepared there from a stock solution of concentration 30 mg/mL and a solution of biological fluid (sodium chloride). To each well, 20 µL of bacterial culture suspension are added. For each bacterial strain, a solution of dimethylsulfoxide (DMSO) was used as a positive control, and as a negative control we used ceftriaxone (C-tri 10). Each microplate is covered and incubated for 24 hours at 37°C. Clear staining of the well indicates no growth and cloudiness of the well indicates bacterial growth. Each experiment was repeated three times.

III. Results and discussion

III.1. Extraction

Extraction rates vary from 1% to 40% for sequential extraction and from 33% to 50% for crude ethanolic extraction. The hexane and ethyl acetate fractions of the roots show the lowest extraction rates (1%). The crude ethanol extract from the bark has the highest extraction rate with 50%. The results reveal the same extraction rate (40%) for the aqueous fractions of barks and roots (Table 1). All these experimental data show that the extraction rate increases with the polarity of the solvent, which could explain the richness of *Bridelia micrantha* in polar compounds. Furthermore, the results also showed that the barks of *Bridelia micrantha* are relatively rich in fatty substances with an extraction rate of 4% for the hexane fraction.

Table 1: Results of bark and root extraction

Parts	Solvents	Volume (ml)	Sample mass (g)	Mass obtained (g)	Extraction rate (%)
Barks	Hexane*	700	101	4.04	4
	AcOEt*			2.02	2
	Ethanol*			20.1	20
	Eau*			40.4	40
	Ethanol**	400	83	41.5	50
Roots	Hexane*	700	150	1.4	1
	AcOEt*			1.5	1
	Ethanol*			19.53	13
	Eau*			60	40
	Ethanol**	400	81	26.72	33

**crude extract, *fraction

III.2. Phytochemical screening

The phytochemical screenings of the bark and root extracts of the plant focused on the families of secondary metabolites presented in Table 2.

The results of the phytochemical screening show that *Bridelia micrantha* is very rich in secondary metabolites such as polyphenols, flavonoids, sterols and polyterpenes, catechols and tannins. The presence of all these

families of compounds can justify the wide use of this plant in traditional medicine for the treatment of several pathologies.

The roots and barks all contain polyphenols, alkaloids, sterols and polyterpenes, which are distributed differently in the solvent extracts. It is noted that the fraction and the crude ethanolic extract of the roots are the richest in secondary metabolites because all the tests are positive except in the case of coumarins and in hexane extract. For the roots, the aqueous extract seems to be the richest qualitatively in secondary metabolites tested. However, we note that the ethanolic extract of the roots contains a high concentration of tannins, unlike the barks.

Table 2.Phytochemical screening of extracts from the bark and root of *Bridelia micrantha*

Organes	Solvents	Polyphenols	Flavonoids	Alkaloids	Sterols& polyterpenes	Catechols	Coumarins	T. Catechic	T. Gallic
Barks	Hexane*	++	-	-	+	-	+	-	-
	AcOEt*	-	+	+	+++	+	-	-	-
	Ethanol*	+	-	+	+	+++	-	+	-
	Aqueous*	++	+	+	+	++	+	+	+
	Ethanol**	+++	-	+	++	+++	-	+	+
Roots	Hexane*	+	-	-	+	-	+	-	-
	AcOEt*	++	+	+	+++	+	-	-	++
	Ethanol*	+++	++	++	+++	+++	-	+++	+++
	Aqueous*	+	+	+	++	++	-	-	++
	Ethanol**	+++	++	+	++	++	-	+	++

+++ strong presence; ++medium presence; + weak presence; - absence ; **crude extract; *fraction

III.3. Antioxidant activity

The antiradical activity of the different extracts was evaluated by their inhibitory capacity on a methanolic solution of $DPPH^{\cdot}$, by measuring the absorbance at a wavelength of 517 nm. Ascorbic acid is used reference. The results of the antiradical activity of the various extracts from the roots and barks of *Bridelia micrantha* are summarized in Table 3 and illustrated in the diagram of Figure 2.

Analysis of the IC50 values of the different extracts of the barks and the roots of *Bridelia micrantha* shows that the ethanolic extracts of the roots and barks have the greatest anti-free radical capacities with respective IC50 values of 0.17 mg/mL and 1.10 mg/mL. This antiradical activity is confirmed by Nwaehujor et al. on the ethyl acetate extract [15] of the roots and by Onoja et al. [16] on the hydroethanolic extract of the bark of the plant. It should also be noted that the ethyl acetate extract of the bark has the highest IC50 (31 mg/mL), and therefore the lowest antioxidant activity. The experimental results show overall that all the root and bark extracts exhibit antioxidant activity with, in particular, IC50s ranging from 0.17 mg/mL to 19 mg/mL. However, it should be noted that for the barks, the hexane extract does not show any antioxidant activity.It is also noted that the ethanolic fractions exhibit greater antioxidant activities than the crude ethanolic extracts. This result could be explained by the existence of unsaturated fatty substances which promote the formation of free radicals or prevent the massive extraction of compounds with antioxidant activity.*Bridelia micrantha* leaf extracts are reported in the literature to possess remarkable antioxidant activity [15-18].

Table 3: IC50 values of barks and roots of *Bridelia Micrantha* in different solvents

Organs	Extract	Inhibitory concentration (CI_{50}) mg/mL
Barks	Hexanic*	-
	Ethylacetate*	31.00 ± 1.77
	Ethanolic*	1.10 ± 0.00
	Aqueous*	14.00 ± 1.48
	Ethanolic**	3.10 ± 0.07
Roots	Hexanic*	19.40 ± 0.14
	Ethylacetate*	5.80 ± 0.04
	Ethanolic*	0.17 ± 0.00
	Aqueous*	0.42 ± 0.00

	Ethanolic**	0.47 ± 0.03
Acide ascorbique		0.082 ± 0.001

-: absence of antioxidant activity, *fractions, **crude extract

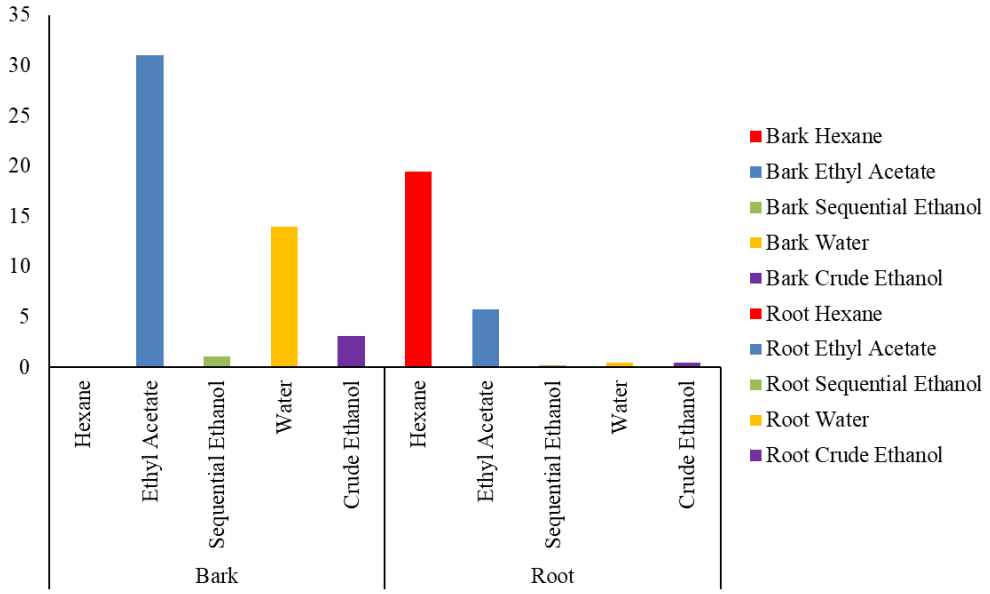

Figure 2. Diagram of the IC50s of barks and roots of *Bridelia micrantha* in different solvents.

III.4. Antibacterial activities

III.4.1. Determination of the sensitivity of different extracts at a concentration of 30 mg/mL on different bacterial strains.

The results show that the strains Escherichia Coli ATCC25922, Enterococcus faecalis ATCC 29213, Staphylococcus aureus ATCC 29212 are sensitive to the ethanolic fraction of the roots with respectively inhibition diameters of 11, 8 and 9 mm (Table 4). Pseudomonas (community strain) is sensitive to the ethanolic extracts of roots and barks with respective diameters of 8 mm and 7 mm (Table 4). The bark extracts are all inactive against *E. Coli*, which is in disagreement with the results of Gangoué-Pieboji et al. [19] who showed that the methanolic extract of bark showed activity against several bacterial strains including *E. Coli*. *Candida albican* shows resistance to all the extracts tested. All strains tested are insensitive to aqueous extracts of roots and barks. Only the ethanolic extracts show antibacterial activities. All the inhibition diameters obtained for the sensitive strains are greater than 4.8 mm for a concentration of 30 mg/mL, which shows the effectiveness of the extracts of *Bridelia micrantha* against the proliferation of bacteria.

Table 4: Table of inhibition diameters obtained during the bacterial susceptibility test

Bacterialstrains	Organs of the plant					
	Roots			Barks		
	Ethanol**	Ethanol*	Aqueous*	Ethanol**	Ethanol*	Aqueous*
Escherichia Coli ATCC25922	na	11	na	na	na	na
Enterococcus faecalis ATCC 29213	na	8	na	na	na	na
Staphylococcus aureus ATCC 29212	na	9	na	na	na	na
Pseudomonas (communitystrain)	7	8	na	na	7	na
Candida Albican ATCC24433	na	na	na	na	na	na

III.4.2. Determination of minimum inhibitory concentrations (MIC) in mg/mL

After the sensitivity tests, it was possible to determine the minimum inhibitory concentrations of the extracts on the strains. For this purpose, a concentration range has been prepared by dilution on well plates and are applied to the strains. The reading is taken after 24 hours of incubation at 37°C and the results obtained are recorded in the table below and shown in Figure 3.

The results obtained show that the MICs of the ethanolic fraction of the roots are respectively 1.875 mg/mL (*E.Coli* and *E.Faecalis*) and 3.75 mg/mL (*S.Aureus* and *Pseudomonas*). Ethanol fractions from roots and bark have the same MIC (3.75 mg/mL) for *Pseudomonas*. The crude ethanol extract of the roots has an MIC of 7.5 mg/mL for *Pseudomonas*. The ethanolic fractions present the lowest MICs, which is in agreement with the antioxidant activities where they have the lowest IC50s and therefore constitute the most active fractions. These antibacterial activities of *Bridelia micrantha* conform well with the traditional use of the roots and barks of the plant for the treatment of bacterial infections such as cough, diarrhea, dysentery, syphilis [20]. The ethanolic fraction of the roots has the lowest IC50 (0.17 mg/mL) and, moreover, it has the greatest antibacterial activity with MICs between 1.875 mg/mL and 3.75 mg/mL.

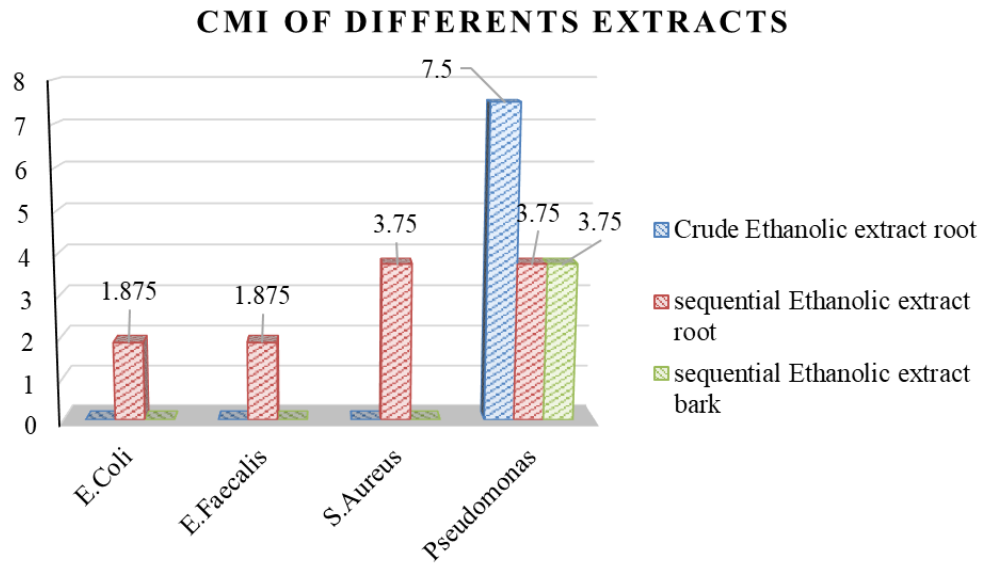

Figure 3. Diagram of mean inhibitory concentrations (MIC in mg/mL)

IV. CONCLUSION

The phytochemical study carried out on extracts from the bark and roots of *Bridelia micrantha* revealed the presence of several families of secondary metabolites such as: polyphenols, alkaloids, sterols and polyterpenes.

The ethanolic fractions show the highest antioxidant activities with respective IC50s of 1.10 ± 0 mg/mL for the roots and 0.17 ± 0 mg/mL for the barks. These fractions show antibacterial activities against certain strains with inhibition diameters greater than 4.8 mm. The evaluation of the antimicrobial activity, through the determination of the MIC showed that the ethanolic fractions constitute the most active part of *Bridelia micrantha*. Considering the importance of this plant in the traditional medicine and its threat of disappearance because of the misuse of the roots, it comes out of our study that the roots can be used in the same way as the leaves and the barks of *Bridelia micrantha* in the treatment of pathologies. However, further study to identify the active principles of each part of the plant is necessary for a good understanding of the mechanisms of action after ingestion.

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IbrahimaDiallo, et. al. "Phytochemical study, determination of the antioxidant and antimicrobial activities of the bark and root extracts of Bridelia micrantha, a plant widely used in traditional African medicine."*IOSR Journal of Applied Chemistry (IOSR-JAC),* 15(10), (2022): pp 08-14.